Journal of Rare Cardiovascular Diseases 2016; 2 (6): 181–184
www.jrcd.eu

ORIGINAL PAPER
Diseases of the heart

Nitric oxide signaling in the ischemic postconditioning of human heart muscle (RCD code: III)

Marcin Kunecki*[1], Wojciech Płazak[1], Piotr Podolec[1], Tomasz Roleder[2], Jolanta Biernat[3], Piotr Kogut[4], Marek A Deja[5], Krzysztof S. Gołba[3]

[1] Department of Cardiac and Vascular Diseases, Institute of Cardiology, Jagiellonian University Medical College, John Paul II Hospital, Krakow, Poland; [2] Department of Cardiology, Medical University of Silesia, Katowice, Poland; [3] Department of Electrocardiology and Heart Failure, Medical University of Silesia, Katowice, Poland; [4] Department of Internal Medicine and Cardiology, H. Klimontowicz Hospital, Gorlice, Poland; [5] Department of Cardiac Surgery, Medical University of Silesia, Katowice, Poland

Abstract

Background: Ischemic heart conditioning is well documented to trigger the intrinsic protective mechanisms of resistance against ischemia/reperfusion (I/R) injury. Previous studies on animal model have suggested that the nitric oxide (NO) mediates the beneficial effect of ischemic postconditioning (POC). We tested the hypothesis that POC provide cardioprotection in the NO-dependent mechanism in the human myocardium. **Methods:** Human atrial trabeculae were subjected to simulated I/R injury. To achieve POC triple brief hypoxia periods were followed by the lethal hypoxia. Non-selective inhibitor of NO synthase: NG-monomethyl-L-arginine (L-NMMA) was used at the time of re-oxygenation in the POC protocol. Contractility of the myocardium was assessed as the maximal force of a contraction (Amax), the rate of rise of the force of a contraction (Slope L) and cardiac muscle relaxation – as the rate of decay of the force of a contraction (Slope T). **Results:** Co-application of L-NMMA with POC resulted in the decrease of Amax, Slope L and Slope T during re-oxygenation period as compared to POC only. **Conclusions:** At re-oxygenation period, the blockade of NO synthesis has deleterious effect on systolic and diastolic function of human myocardium, as well as attenuates the beneficial effect of ischemic POC. JRCD 2016; 2 (6): 181–184

Key words: ischemic heart disease, reperfusion, cardioprotection, nitric oxide synthase

Background

Ischemic heart disease have been remarked as leading cause of morbidity and mortality in developed countries. The early restoration of coronary flow is crucial to reduce ischemic myocardial damage due to acute vessel occlusion. However, reperfusion has the potential to exacerbate lethal tissue injury causing increase of the infarct area of the myocardium by up to 50% of the final size [1]. This mechanism known as ischemia/reperfusion (I/R) injury manifests as the decrease of potential benefits of reperfusion. The mechanisms underlying postconditioning (POC) are still not clarified, but there is an experimental evidence with the animal models, that nitric oxide (NO) may be the part of the endogenous cardioprotective response to I/R injury. Sequences of brief ischemia periods applied before (preconditioning) or after (POC) coronary occlusion are well documented to trigger protective mechanisms to the heart muscle against I/R injury in *in vitro* [2,3] and *in vivo* studies [4–8]. The *ex vivo* studies indicate that POC protects human myocardium through the activation of the reperfusion injury salvage kinases (RISK) pathway and inhibition of elements of RISK pathway: MEK1/2 and PI3K abolishes the recovery of contractile function conferred by POC [3]. We hypothesized that ischemic POC provides cardioprotection in human heart muscle through NO-dependent pathway what may give insight into the explanation of the protective mechanisms against I/R injury.

Methods

The experiments were performed on muscular trabeculae obtained from the right heart atrial appendages of 58 consecutive patients (35 males/23 females) subjected to the coronary artery bypass

Conflict of interest: none declared. Submitted: February 18, 2016. Accepted: March 17, 2016.

* Corresponding author: Department of Cardiac and Vascular Diseases, Institute of Cardiology, Jagiellonian University Medical College, John Paul II Hospital, Krakow, Poland, Prądnicka str. 80, 31-202 Krakow, Poland; tel. 0048 12 614 22 87, fax 0048 12 423 43 76; e-mail: mkunecki@op.pl

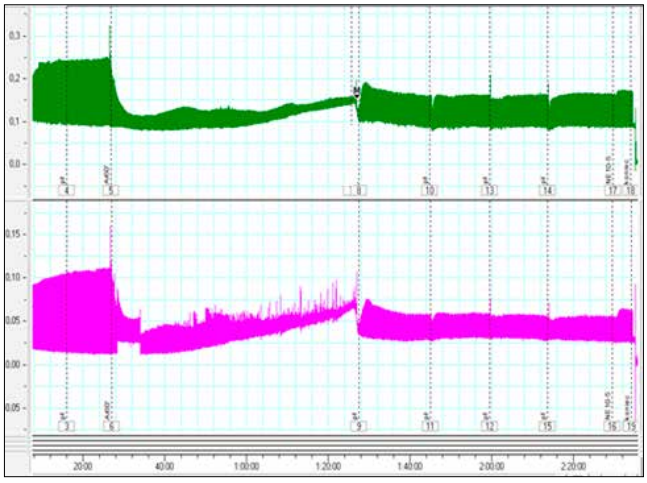

Figure 1. Time course of the effect of nitric oxide modulation observed in the absence (top tracing – Control protocol) and the presence of NG-monomethyl-L-arginine (L-NMMA) (bottom tracing – L-NMMA protocol). Notice that the amplification was reduced during hypoxic period. During reoxygenation period improvement of contractility is significant higher in the Control protocol

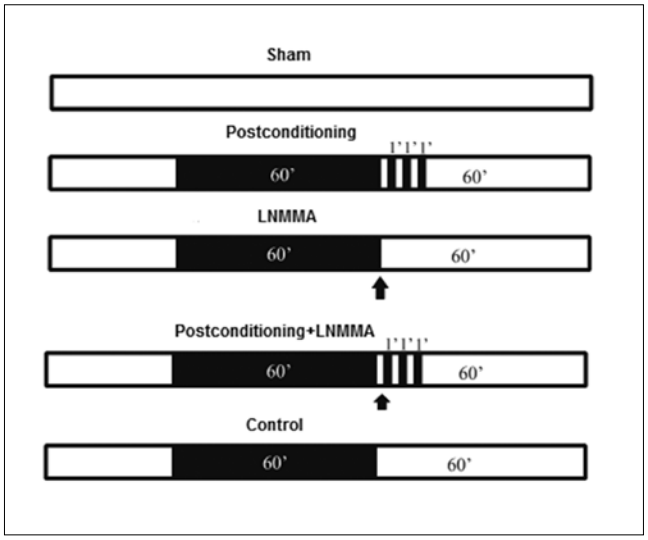

Figure 2. Experimental protocols: Sham – normoxic conditions, Postconditioning – triple brief hypoxia periods followed the 60' lethal hypoxia, NG-monomethyl-L-arginine (LNMMA) – hypoxic conditions with using LNMMA at the moment of reoxygenation, Postconditioning+LNMMA – co-application of postconditioning protocol and LNMMA, Control – 60' hypoxic condition followed the 60' of reoxygenation

surgery. Patients diagnosed with the significant valvular heart disease or with severe heart failure therapy were excluded from the study. There were no significant differences in age, sex and pharmacotherapy between the patients from which the trabeculae were taken and were subjected to each experimental protocol. Non selective inhibitor of NO synthase: NG-monomethyl-L-arginine (L-NMMA) was obtained from Sigma Aldrich Co. Norepinephrine was obtained from Polfa Warszawa. The Local Bioethics Committee approval for the use of human tissue was obtained and individual patient consent was waived. All experiments were performed according to the principles stated in the Declaration of Helsinki.

The fragments of the human right heart atria were transported from the cardiac surgery room to the laboratory in an ice-cold Krebs-Henseleit solution ([M]: NaCl 118.0, KCl 4.70, CaCl2 1.52, MgSO4 1.64, NaHCO3 24.88, KH2PO4 1.18, glucose 11.0, and sodium pyruvate 2.0; pH 7.4). Two muscular trabeculae, each less than 1 mm in diameter, were dissected from the right heart atria and incubated in 2 separate organ baths (Schuler Organbath, Hugo Sachs Elektronik, March-Hugstetten, Germany [HSE]) both filled with Krebs-Henseleit solution warmed up to 37°C. Two trabeculae from each patient were every time studied simultaneously and exposed to hypoxia protocol including: 60 min of hypoxia (incubation in Krebs-Henseleit buffer deprivated of glucose and pyruvate and saturated with 95% argon and 5% carbon dioxide) with subsequent 60 min of re-oxygenation (incubation in Krebs-Henseleit buffer saturated with the 95% oxygen and 5% carbon dioxide). The buffer was replaced every 15 min, except the time of hypoxia. Every trabecula was stretched to 90% of its optimal tension strength, according to the Frank-Starling relationship and all trabeculae were driven throughout experiments with 1 Hz 50 ms square stimuli using platinum field electrodes and a stimulator (Type 215, HSE). The systolic function of every trabecula was recorded with the use of F30 isometric force transducer (Type 372, HSE). The signal was enhanced with a bridge amplifier (Type 336, HSE) and recorded by a PowerLab/4SP system and analyzed off-line using Chart software (AD Instruments, Chalgrove, Oxfordshire, UK). Sample time course of the function of trabeculae is presented in figure 1. Each experimental protocol was completed with 10 μM of norepinephrine (NE) application to assess the viability of trabeculae.

POC protocol consisted of the sequence of 1-min hypoxia with subsequent re-oxygenation both repeated 3 times applied at the beginning of 60 min re-oxygenation. The number of the ischemic cycles was based on previously published data [7,8]. To determine the effects of modulation of NO pathway, the non-selective inhibitor of NO synthase: L-NMMA 10^{-4}M was used at the time of re-oxygenation (L-NMMA protocol) or added to POC protocol at the time of reoxygenation (POC+L-NMMA protocol). The trabeculae in Control group were subjected to hypoxia protocol only. Additional trabeculae were exposed to 120-min non-hypoxic stimulation in the sham protocol (Sham). The experimental protocols are depicted in figure 2.

The contractility of the myocardium assessed as the maximal force of a contraction (Amax), the rate of rise of the force of a contraction (Slope L) and cardiac muscle relaxation – as the rate of decay of the force of a contraction (Slope T) were obtained in 10^{th}, 15^{th}, 30^{th}, 45^{th} and 60^{th} min of re-oxygenation and after the NE application.

Data analysis

The results are presented as the percent of values obtained before experimental protocol application. All continuous data are presented as a mean ± standard error of the mean (SEM). Two-way

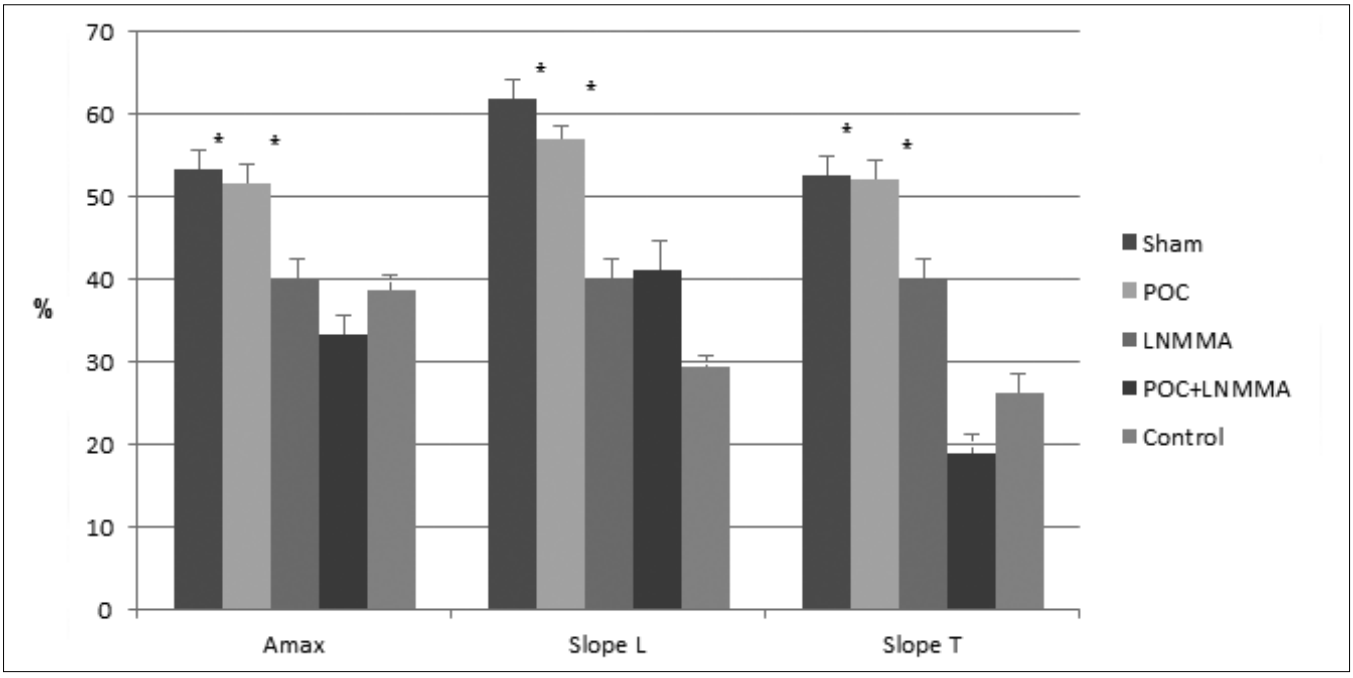

Figure 3. The effect of postconditioning and nitric oxide synthase modulators on function of human myocardium during the re-oxygenation period. Figures present analysis of maximal force of a contraction (Amax), the rate of rise of the force of a contraction (Slope L) and cardiac muscle relaxation – as the rate of decay of the force of a contraction (Slope T). Data are presented as mean±SEM and compared by two way analysis of variance with Holm-Sidack's post hoc test. *p<0.05 marked as significantly different (Sham or POC vs. L-NMMA, POC+L-NMMA, Control protocols)

analysis of variance (ANOVA) with Holm-Sidack's test was used to compare the results of values from 10th to 60th min of re-oxygenation. *p* values less than 0.05 were considered statistically significant. Statistical analysis was performed using SigmaPlot software (ver. 10.0.1.2. **Systat Software Inc.** San Jose, CA, USA).

Results

All contractive and relaxative parameters were significantly higher for non-hypoxic stimulation (Sham) as compared to POC+L-NMMA, L-NMMA, Control protocols during re-oxygenation period and after NE application.

All contractive and relaxative parameters were significantly higher during reoxygenation period for POC as compared to POC+L-NMMA, L-NMMA, Control protocols during re-oxygenation period and after NE application. We observed non-significant differences of results in Sham and POC protocols. All detailed results are included in figure 1 and 3.

Discussion

Previous studies utilized animal models to describe the role of NO signaling pathway in POC-induced cardioprotection. Our study is the first to assess this process in hypoxic human myocardium in vitro. We observed that POC protocol significantly protected the human myocardium against simulated I/R injury. The main finding of our study is that beneficial effect of POC is NO-dependent and blockade of NO-synthase abolished the cardioprotective effect of POC in human heart muscle. Our study was performed on isolated fragments of human right atria. The *ex vivo* experimental model let to avoid the influence of confounding factors, like the effect of drugs or the presence of collateral circulation, what is important in case of *in vivo* studies. In this model we did not assess the infarct size, but the differences of contractility as a functional consequences of cardiac ischemia.

In 2003, Zhao et al. described a phenomenon another endogenous myocardial protection – POC. This time the intervention was based on the use of short-term cyclic episodes of ischemia and re-oxygenation in the first minutes of reperfusion [9].

Many investigators have studied these phenomena and many of autacoids were examined to be the endogenous trigger of pre- and POC. NO appears to be a common mediator of the protection elicited by pharmacological and non-pharmacological interventions. Previous data showed that POC-mediated cardioprotection is related to the activity of the NO synthase and depends on NO concentration [10,11]. Although NO at low concentration triggers the cardioprotection of human heart, its protective capacity is lost at high concentration, due to the increase of free radicals concentration, especially the peroxynitrite [12]. NO seems to be the common element of intracellular signaling pathway of G-protein coupled receptor ligands, e.g. opioids, adenosine, bradykinin. These factors may mimic the effect of ischemic pre- and POC. The crucial role of the cardioprotective effect of all pharmacological and non-pharmacological interventions is maintaining the mitochondrial permeability transition pores (mPTP) closed during reperfusion period. Mitochondrial ATP-sensitive potassium channels (mKATP) control the activity of the mPTPs [13]. NO in low concentration

was documented to be a potent mKATP opener through activation of anti-apoptotic cascade of enzymes called RISK pathway [14-17].

Tsang et al. have found that POC induces eNO synthase phosphorylation and the inhibitor of RISK pathway element – phosphoinositide 3-kinase, reduces the eNO synthase activation and abrogates the cardioprotective effect of POC [18]. Similarly, Yang et al. demonstrated that NO synthase inhibition with L-NMMA may also abrogate the effect of POC, suggesting that NO synthase may be required for cardioprotection [19]. This data implicates NO, especially NO synthase, as a relevant for POC-induced cardioprotection.

Conclusion

At re-oxygenation period, the blockade of NO synthesis has deleterious effect on systolic and diastolic function of human myocardium, as well as attenuates the beneficial effect of ischemic POC.

References

1. Hausenloy DJ, Yellon DM. Preconditioning and postconditioning: new strategies for cardioprotection. Diabetes Obes Metab 2008;10:451–459.
2. Ghosh S, Standen NB, Galinanes M. Preconditioning the human myocardium by simulated ischemia: studies on the early and delayed protection. Cardiovasc Res 2000; 45: 339–350.
3. Sivaraman V, Mudalagiri NR, Di Salvo C et al. Postconditioning protects human atrial muscle through the activation of the RISK pathway. Basic Res Cardiol 2007; 102: 453–459.
4. Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. Lancet 1993; 342: 276–277.
5. Alkhulaifi AM, Yellon DM, Pugsley WB. Preconditioning the human heart during aorto-coronary bypass surgery. Eur J Cardiothorac Surg 1994; 8: 270–275.
6. Staat P, Rioufol G, Piot C et al. Postconditioning the human heart. Circulation 2005; 112: 2143–2148.
7. Luo W, Li B, Chen R, et al. Effect of ischemic postconditioning in adult valve replacement. Eur J Cardiothorac Surg 2008; 33: 203–208.
8. Halkos ME, Kerendi F, Corvera JS et al. Myocardial protection with postconditioning is not enhanced by ischemic preconditioning. Ann Thorac Surg 2004; 78: 961–969.
9. Zhao ZQ, Corvera JS, Halkos M,et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 2003; 285: H579-H588.
10. Lochner A, Marais E, Genade S, et al. Nitric oxide: A trigger for classic preconditioning? Am J Physiol Heart Circ Physiol 2000; 279: H2752–H2765.
11. Penna C, Cappello S, Mancardi D et al. Post-conditioning reduces infarct size in the isolated rat heart: Role of coronary flow and pressure and the nitric oxide/cGMP pathway. Basic Res Cardiol 2006; 101: 168–179.
12. Trochu JN, Bouhour JB, Kaley G, et al. Role of endothelium-derived nitric oxide in the regulation of cardiac oxygen metabolism: implications in health and disease. Circ Res 2000;87: 1108–1117.
13. Zalewski J, Nessler J. The endogenous cardioprotection during reperfusion in acute myocardial infarction. Kardiol Pol. 2011; 69 Suppl 3: 67–74.
14. Headrick JP, See Hoe LE, Du Toit EF, et al. Opioid receptors and cardioprotection –'opioidergic conditioning' of the heart. Brit J Pharm 2015; 172 2026–2050.
15. Correa F, Buelna-Chontal M, Chagoya V, et al. Inhibition of the nitric oxide/cyclic guanosine monophosphate pathway limited the cardioprotective effect of post- conditioning in hearts with apical myocardial infarction. Eur J Pharmacol 2015; 765: 472–481.
16. Folino A, Losano G, Rastaldo. Balance of Nitric Oxide and Reactive Oxygen Species in Myocardial Reperfusion Injury and Protection. J Cardiovasc Pharmacol 2013; 62: 567–575.
17. Roleder T, Gołba KS, Kunecki M,et al. The co-application of hypoxic preconditioning and postconditioning abolishes their own protective effect on systolic function in human myocardium. Cardiol J 2013, 20: 472–477.
18. Tsang A, Hausenloy DJ, Mocanu MM, et al. Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase- Akt pathway. Circ Res 2004; 95: 230–232.
19. Yang X, Downey JM, Cohen MV. Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by activation of ERK and production of nitric oxide. Circulation. 2003; 108 (suppl IV): 158.